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Brief communication

Locomotor and pyretic effects of MDMA–ethanol associations in rats

Jean-Christophe Cassel^{a,*}, H  l  ne Jeltsch^a, Julie Koenig^a, Byron C. Jones^b^a*Laboratoire de Neurosciences Comportementales et Cognitives, UMR 7521 CNRS – Universit   Louis Pasteur, IFR 37 Neurosciences, 12 rue Goethe, F-67000 Strasbourg, France*^b*Biobehavioral Health, 315 E. HHD Building, The Pennsylvania State University, University Park, PA 16802, USA*

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Abstract

3,4-Methylenedioxymethamphetamine [(MDMA) or ecstasy] is a popular club drug often used in combination with ethanol. In the current study, we investigated the effects of MDMA and ethanol combinations on locomotor activity and body temperature of rats. For four consecutive days, male Long–Evans rats were treated daily with a 10-mg/kg dose of MDMA with or without a 1.5-g/kg dose of ethanol. 3,4-Methylenedioxymethamphetamine increased spontaneous activity (on average +1,140%), and this increase was potentiated by ethanol on all days (on average +1,710%). Moreover, ethanol inhibited the MDMA-induced hyperthermia (on average –1.3  C) by the first day of treatment, but not on subsequent treatment days, supporting the suggestion that this effect may undergo tolerance. These observations seem to indicate that combined ethanol–MDMA may induce effects on locomotor activity and thermoregulation that involve separate mechanisms, the first one being less sensitive to tolerance than the second one might be. Results of our study have important implications for understanding the motivation and the health risks of polydrug abusers combining ecstasy and ethanol.    2005 Elsevier Inc. All rights reserved.

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1. Introduction

The amphetamine derivative (\pm)-3,4-methylenedioxymethamphetamine [(MDMA), ecstasy] has become one of the most popular recreational drugs of abuse among young people, particularly in the club culture [see, for example, Green et al. (1995, 2003) and Schifano (2004)]. In the short term, MDMA induces release of serotonin and dopamine in the brain, causes a dose-dependent increase in spontaneous physical activity, and causes a hyperthermia that may be fatal in rodents, primates, and human beings (Schifano, 2004). In the long term, single or multiple high doses of MDMA may also result in serotonergic toxicity in brain regions, such as the hippocampus, cortex, and striatum (Green et al., 2003). The combination of MDMA with various other drugs, such as amphetamines, cocaine, cannabis, and perhaps predominantly ethanol, is a frequent pattern of MDMA use in human

beings, presumably to boost its effects [see, for example, Lora-Tamayo et al. (2004), Pedersen and Skrandal (1999), and Schifano (2004)]. Moreover, ethanol is known to be one of the drugs most commonly associated with MDMA [see, for example, Lora-Tamayo et al. (2004), Pedersen and Skrandal (1999), and Schifano (2004)]. Until recently, to our knowledge no peer-reviewed journal has published experimental work addressing the effects of acute ethanol interaction with MDMA on spontaneous activity or other physiologic changes such as body temperature alterations in animals. The only published articles we know about concerning the effects of MDMA and ethanol interactions are focused on physiologic or psychologic consequences in human beings: one on the immune system (Pacifci et al., 2001) and the other on psychomotor and subjective effects of the drug combination (Hern  ndez-Lopez et al. 2002). In this article, on the basis of two approaches, we demonstrate that ethanol co-administration potentiates the hyperlocomotion induced by MDMA but prevents its hyperthermic effects. We also show that the preventive effects of ethanol on MDMA-induced hyperthermia disappear when what appears to be tolerance to ethanol develops in the rats. These observations might have important implications for the health risks of polydrug abusers who combine ecstasy and ethanol.

* Corresponding author. LN2C, UMR7521, CNRS/Universit   Louis Pasteur, 12 rue Goethe, F-67000 Strasbourg, France. Tel.: +33-390-241-952; fax: +33-390-241-958.

E-mail address: jean-christophe.cassel@psycho-ulp.u-strasbg.fr (J.-C. Cassel).

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2. Materials and methods

2.1. Subjects

All procedures were conducted in conformity with the institutional guidelines (council directive 87848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale; permission 6212 to J.-C.C. and 6714-bis to H.J.; NIH publication, 86-23, revised 1985). Adult, male, Long-Evans rats (3 months of age; CERJ, Saint-Berthevin, France) were housed individually in transparent Makrolon cages (42 × 26 × 15 cm) under controlled temperature (23°C) and a 12-h light/12-h dark cycle (lights on at 7:00 a.m.). The rats were housed individually because we used them only over a relatively short period. In addition, grouping males generates fighting, which might be a potential source of stress and may interfere with responses to MDMA. The rats were allowed to acclimatize to the laboratory conditions for 1 week before the experiment on body temperature was started and 2 weeks before the activity recordings were performed (see below).

2.2. Drugs

Ethanol [20%, weight/volume] was prepared from absolute ethanol diluted in 0.9% sodium chloride solution and injected intraperitoneally at a dose of 1.5 g/kg. Intraperitoneal administration of a 1.5-g/kg dose of ethanol typically results in a blood ethanol concentration of about 175 mg/dl. (\pm)-3,4-Methylenedioxymethamphetamine (National Institute on Drug Abuse, Bethesda, MD, USA) was diluted in 0.9% sodium chloride solution and injected intraperitoneally at a dose of 10 mg/kg. The drugs, whether administered alone or in combination, were injected in a volume of 7.5 ml/kg, 30 min before the first temperature measures, or 1 to 5 min before activity recording was started. For the combined administration, MDMA was dissolved in the 20% ethanol solution. The dose of MDMA used in the current study was chosen on the basis of results of preliminary experiments, in which we found that a single injection of 20 mg/kg was lethal in 90% of rats [although the 50% lethal dose (LD₅₀) generally reported for rats in the literature is about 50 mg/kg]. A dose of 5 mg/kg induced effects comparable to those reported in this article after 10 mg/kg, but their magnitude and duration were smaller.

On four occasions, 24 h apart, rats were treated with one of the drugs/combination of drugs for assessment of either locomotor activity or body temperature measurements.

2.3. Locomotor activity

Spontaneous activity of the rats was measured in their home cage, and all rats were tested at once. No experimenter entered into the room in which activity was measured during recording. The cages were taken from the colony room and placed on shelves (eight cages per shelf) in a separate room (light conditions as in the colony room). Rats

had free access to food and water during activity recording. Each cage contained two crossing infrared light beams targeted on two photocells, 4.5 cm above floor level and 28 cm apart. The number of crossings in the cage (successive interruptions of the beams, and thus only two-dimensional movements) was monitored continuously by a microcomputer. Activity was first monitored continuously during the 4 days preceding the first drug administration (data not shown) to habituate the rats to the conditions of the test room. After each drug administration, the activity was recorded continuously for 6 h. The drugs were injected at 12:00 noon. The ambient temperature during measurements of locomotor activity was 25°C \pm 0.1°C, thus about two degrees higher than in the colony room. This difference was due to a fortuitous disturbance of temperature control on the first day, and we decided to keep that value for the three other days to ensure as stable as possible experimental conditions for all activity recording. The final group sizes were as follows: saline, $n = 9$; ethanol, $n = 10$; MDMA, $n = 8$; and ethanol + MDMA, $n = 8$. From the initial 10 rats of the MDMA and ethanol + MDMA groups, 2 rats had died in each group after the first MDMA injection. These rats were different from those used for body temperature measurements.

2.4. Body temperature

With the exception of the occasions of temperature measurements, rats had free access to food and water. Observation of the rats' behavior in their home cage was possible between each series of temperature measurements. Rectal temperature was measured with a Pic indolor Vedo Flex (Artsana-Grandate, Italy) digital thermometer with a 0.1°C precision, and the probe was lubricated with petroleum jelly (Vaseline). Determination of the temperature took a maximum of 30 s. The first measurement was taken 1 h before drug treatment (between 11:00 and 11:20 a.m.). The other measurements were made 30, 60, 120, 180, and 300 min after drug administration. The ambient temperature during measurements was 23°C \pm 0.1°C. Between measurements, the rats remained in their home cage. The final group sizes were as follows: saline, $n = 5$; ethanol, $n = 6$; MDMA, $n = 5$, and ethanol + MDMA, $n = 7$. One rat from the MDMA group died after the first injection.

2.5. Statistical analyses

All data were evaluated by using analysis of variance (ANOVA), followed, where appropriate, by multiple comparisons with the use of the Newman-Keuls multiple range test (Winer, 1971). Analysis of body temperature was performed on the temperature changes according to the predrug temperature. *T* tests for paired samples were also used for comparison of average temperatures on days 1 and 2 within each experimental group.

3. Results

3.1. Locomotor activity

The basal locomotor activity for all rats had been recorded over the first 3 of 4 days of acclimatization to the experimental conditions. There was no significant difference among the four groups, in terms of either diurnal or nocturnal spontaneous home cage activity on each of these days (data not illustrated). Analysis of the activity scores found on the 6 h that followed the injection (Fig. 1) showed a significant overall treatment effect on day 1 [$F(3, 30) = 14.1, P < .001$], day 2 [$F(3, 30) = 25.5, P < .001$], day 3 [$F(3, 30) = 15.5, P < .001$], and day 4 [$F(3, 30) = 14.6, P < .001$]. On day 1, multiple comparisons showed that the treatment effect was due to a significantly higher activity in MDMA-treated rats in comparison with their saline-treated or ethanol-treated counterparts ($P < .05$), as well as in ethanol + MDMA-treated rats in comparison with each of the three other groups ($P < .05$). The difference between saline-treated and ethanol-treated rats was not significant ($P = .99$). On days 2 and 4, the same between-group differences were found. On day 3, the only difference from

the other 3 days was that the difference between MDMA-treated and ethanol + MDMA-treated rats was not significant ($P = .27$). When the overall activity scores accumulated over days were compared among groups (see inset, Fig. 1), we found that ethanol + MDMA-treated rats were significantly more active than rats of the three other groups ($P < .01$), and that MDMA-treated rats were more active than saline-treated or ethanol-treated rats ($P < .001$). The difference between saline-treated and ethanol-treated rats was not significant ($P = .99$). In summary, ethanol potentiated the MDMA-induced hyperactivity.

3.2. Body temperature

Results for analysis of body temperature are displayed in Fig. 2. For the rats to be injected with saline, the average basal body temperature (computed over 4 days) 60 min before the injections was $37.3^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ and $37.7^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ in rats that subsequently received saline and ethanol, respectively; $37.7^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ in those to be given MDMA; and $37.8^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ in those to be given ethanol + MDMA. There was no significant difference among the four groups on this variable [$F(3, 19) = 2.6$]. On the first day of injection after MDMA treatment, the temperature typically had reached

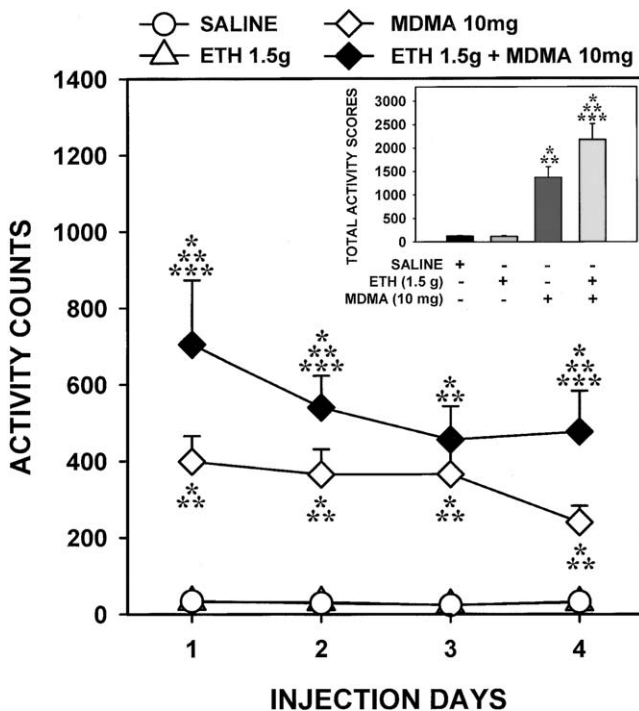


Fig. 1. Locomotor activity recorded over 6 h after intraperitoneal injection of a 10-mg/kg dose of 3,4 methylenedioxymethamphetamine (MDMA), and potentiation of this response by co-administration of a 1.5-g/kg dose of ethanol (ETH). Injections occurred on four consecutive days, 24 h apart. Inset shows the activity cumulated over the 4 days (underneath the abscissa, “+” indicates the treatment administered). Means \pm standard error of the mean are shown. *Indicates a significant difference in comparison with saline; **indicates a significant difference in comparison with ethanol, $P < .05$; and ***indicates a significant difference in comparison with MDMA, $P < .05$.

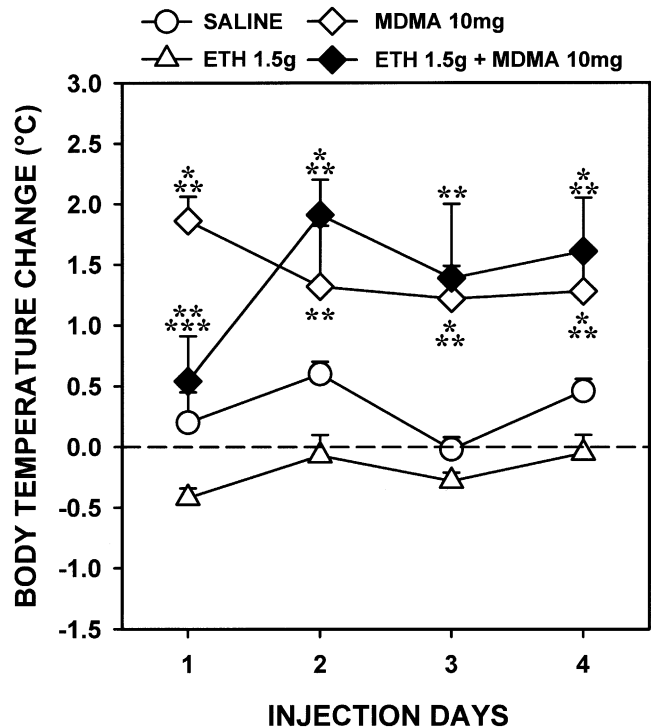


Fig. 2. Body temperature changes recorded 60 min after intraperitoneal injection of a 10-mg/kg dose of 3,4 methylenedioxymethamphetamine (MDMA), and reversal of the MDMA-induced hyperthermia by co-administration of a 1.5-g/kg dose of ethanol (ETH) on the first injection day. Means \pm standard error of the mean are shown. *Indicates a significant difference in comparison with saline; **indicates a significant difference in comparison with ethanol, $P < .05$; and ***indicates a significant difference in comparison with MDMA, $P < .05$.

a maximal value 60 min after drug injection ($+1.9^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$). The temperature had returned to near-normal values at the postinjection delay of 300 min. It had decreased only slightly after 120 min. After ethanol injection, the temperature decreased by about half a degree ($-0.4^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$) and was still close to this value after 300 min ($-0.5^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$). (These findings are not illustrated in this article.) It may be worth mentioning that from the observations of the behavior of the rats in their home cage between two measurement series, it appeared that the behavior of MDMA-treated and ethanol + MDMA-treated rats was similar. The main changes observed were quantitative only.

Analysis of body temperature changes found 60 min after injection showed a significant overall treatment effect on day 1 [$F(3, 19) = 12.01, P < .001$], day 2 [$F(3, 19) = 9.4, P < .01$], day 3 [$F(3, 19) = 4.6, P < .05$], and day 4 [$F(3, 19) = 7.7, P < .01$]. On day 1, multiple comparisons showed that the treatment effect was due to an increase in temperature in MDMA-treated rats in comparison with findings for the three other groups ($P < .01$), as well as in ethanol + MDMA-treated rats in comparison with findings for rats treated with ethanol alone ($P < .05$). The difference between saline-treated and either ethanol-treated or ethanol + MDMA-treated rats was not significant ($P = .12$ and $.37$, respectively). On day 2, MDMA-treated and ethanol + MDMA-treated rats no longer differed significantly from each other ($P = .17$). However, the temperature increase in ethanol + MDMA-treated rats was significantly different from the changes found in ethanol-treated or saline-treated rats ($P < .05$), and that of MDMA-treated rats was different from the change found in ethanol-treated rats ($P < .05$). On day 3, the picture was the same, except that only the difference between ethanol + MDMA-treated and saline-treated rats showed a trend ($P = .06$), and MDMA-treated rats now differed significantly from their saline-treated counterparts ($P < .05$). On day 4, the difference between ethanol + MDMA-treated and saline-treated rats was, again, significant ($P < .05$). Results of paired samples t tests showed that the only significant change on day 2 in comparison with day 1 was in ethanol + MDMA-treated rats ($t = 4.01, P < .01$). In ethanol-treated rats, there was a strong tendency ($t = 2.24, P = .07$). In summary, ethanol prevented the hyperthermia induced by MDMA on the first injection day, but not on the three subsequent days.

4. Discussion

Findings of the current study confirm that MDMA treatment induces both hyperlocomotion and hyperthermia in rats [see also, for example, Cole and Sumnall (2003) and Mechan et al. (2002)]. To the best of our knowledge, there is no published report on experimental examination of the effects of ethanol and MDMA in combination in animals. We have shown that ethanol acts synergistically with MDMA to increase locomotor activity in rats, even when adminis-

tered repeatedly. In addition, ethanol ameliorates the hyperthermia produced by MDMA, but only on the first of consecutive daily injections. It may be noteworthy that both these ethanol-induced effects have been replicated in an ongoing experiment in our laboratory (unpublished observations, J.-C. Cassel, H. Jeltsch, S. Ben Hamida, J. Koenig, C. Kelche, and B. C. Jones, 2004), indicating that the data reported herein are robust. It is interesting that, on the first day, ethanol potentiated the locomotor effects of MDMA, whereas it reduced the MDMA-induced hyperthermia. This finding seems to indicate that this hyperthermia was not a consequence or a correlate of hyperactivity.

Our observations on both activity and body temperature are important in that they reveal that the combination of MDMA and ethanol may have unexpected consequences. The hyperactivity produced by MDMA taken, for example, at a rave party may be greatly increased when accompanied by ethanol, which can be related to the findings on subjective effects in human beings reported by Hernández-Lopez et al. (2002). In addition, the apparent protection against the hyperpyretic effects of MDMA by ethanol may disappear on subsequent administrations given in short intervals. The exact mechanisms of both effects are currently unknown to us. In rats, ethanol does not cause hyperactivity as it does in mice [see, for example, Cohen et al. (1997)]. It is, however, worth mentioning that this synergism is in line with results that Pacifici et al. (2001) and Hernández-Lopez (2002) obtained in studies with human subjects. These investigators showed that physiologic and psychopathologic effects of MDMA can be increased in the presence of ethanol. A possible explanation might be that ethanol, for some reasons (e.g., facilitation of the blood–brain barrier permeability toward MDMA metabolites), has increased MDMA exposure at the brain level. However, this possibility would suppose that all effects of MDMA are increased in the presence of ethanol, which was obviously not the case with body temperature in the current study.

As far as that hyperthermia may participate in the neurotoxic effects of MDMA, the combined effects on hyperthermia pose perhaps an even greater risk. If, on the first occasion of the use of MDMA, one consumes ethanol, the individual may indeed be protected against neurotoxic or even fatal effects. On the second occasion, one may have become tolerant to the apparent protective effects of ethanol without concomitant tolerance to the hyperpyretic effect of MDMA. Tolerance to ethanol may develop rapidly, and it has been demonstrated that tolerance to the hypothermic effect of ethanol may appear as soon as a second exposure to the drug in a delay of 24 or 48 h after the first one [see, for example, Chan and York (1994) and Khanna et al. (1993)]. In fact, ethanol is not so much an agent of hypothermia as an agent that produces poikilothermia (Myers, 1981). Thus, in a very warm environment (e.g., a rave party in a very warm room, or even in a hot tub), ethanol may increase the risk of a fatal hyperthermic crisis. This issue will be addressed in future experiments.

5. Conclusions

In a recent review, Cole and Sumnall (2003) mentioned that young people who use ecstasy may also associate other drugs to protect against the neurotoxic effects of MDMA. Given that hypothermia may be neuroprotective [see, for example, Colado et al. (2001)], and if the expected protection involved hypothermic effects of ethanol or prevention of MDMA-induced hyperthermia, findings of the current study seem to indicate that this might be true when ethanol and MDMA are associated for the first time, but no longer true on subsequent associations, nor after tolerance to ethanol has developed. In Western countries, in which many human beings are exposed to small quantities of ethanol in early/middle adolescence, it is possible, and even probable, that preexisting tolerance to ethanol mitigates this protection against the hyperthermic effects of MDMA *ab initio*. Results of the current study demonstrate that combined ethanol–MDMA may induce effects on activity and thermoregulation that involve separate mechanisms, the first one being less sensitive to tolerance than the second one.

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